CONCERNING THE PIGMENTS OF THE TWO-SPOTTED OCTOPUS AND THE OPALESCENT SQUID [1]

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The majority of fishes and invertebrate species of the sea exhibit integumentary colors due either to melanin pigments or, especially in the invertebrates, to carotenoids. Carotenoids are often also relatively concentrated in such tissues as the liver or digestive gland, and in eggs. Chromoproteins, flavines, purines, and certain more rare pigmentary compounds are discussed by numerous writers (e.g. Verne, 1926).

This paper concerns the pigments encountered in various tissues of two cephalopods of Pacific waters, namely the two-spotted octopus, *Paroctopus bimaculatus,* and the common squid, *Loligo opalescens.* Cephalopods studied by Lönnberg (1935) and the two species investigated by us have revealed a striking general absence of carotenoids from the integument and gonads and only traces in the eyes, but there are considerable quantities of these yellow, orange, or red pigments in the "liver" [2] of *Paroctopus.* Melanins characterize the ink of these mollusks, and are present also in the iris and skin. Octopods show relatively large quantities of melanin in their highly specialized chromatophores, while certain squids exhibit very little in the integument, the pigment there often appearing light brown or reddish instead of grey or deep brown as in the former group. We encountered, in the pericardial glands of the octopus, small amounts of yellow flavines as well as a red water-soluble pigment of unknown identity in the kidneys.

Lonnberg (op. cit.), making preliminary studies of the carotenoids of three cephalopods, reports lutein in the eyes of *Sepiola scandica, Rossia macrosoma,* and *Eledone cirrosa.* He found no carotenoids in the eggs of *Rossia* or *Sepiola,* and only possible traces in the testes of *Eledone.* Extracts of *Rossia* eggs, however, gave a strong color reaction with $SbCl_3$, suggesting the possible presence of vitamin A or a similar com-

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[2] The term as employed here refers to the large red-brown, grey or greenish gland communicating by a pair of ducts with the alimentary canal at the junction of stomach and intestine. Actually the "liver" and "pancreas" are incorporated together, without a very sharp division, in the single gland.

pound. Carotenoids were not detected in the skin of *Sepiola* or *Rossia,* but were reported in the mantle of *Eledone.* Especially notable was the presence of quantities of a carotenoid resembling lutein in the liver of *Eledone.* The livers of the other two species were not given special study.

Escher-Desrivières, Lederer and Verrier (1938) cite older observations of Kruckenberg on the purple pigments in retinae of certain cephalopods. Studying the retinal pigments of *Sepia officinalis, Octopus vulgaris,* and *Eledone moschata,* these authors found certain chemical and spectroscopic similarities between the cephalopod eye-pigments and those of classical types. They found, however, that the cephalopod retinal purple was stable in light, and they failed to find A vitamins in the material. More recent investigations by Wald (1941) have demonstrated vitamin A and retinene in the retina of the squid *Loligo pealii,* but no traces of these or other carotenoids in other squid tissues. According to Wald, vertebrates and invertebrates alike use vitamins A in the eye. Failure by other investigators to identify these carotenoids in the eyes of squids and other invertebrates is attributed by Wald to the low capacity of such forms for storing these substances. Wald points out that the quantity of vitamin A remains constant in the squid's eye in light and darkness, and that it may not participate directly in the visual processes. He claims that the deep purple pigment of the squid retina, sometimes referred to as visual purple, is really "a photostable, alkali soluble probably melanoid pigment," acting as a light-screen for the retinal cells, but probably playing no role in vision proper.

Materials and Methods

Specimens of *Paroctopus bimaculatus* were taken freshly from the sea and killed by immersing in tap water just preceding analysis. Small specimens (about 6 inches in length) of the squid, *Loligo opalescens,* were bought from wholesale fish-dealers in San Diego. The animals showed no signs of decomposition, having been stored on ice since capture a few days earlier.

Brown or black melanin pigments were detected in the various tissues merely by visual inspection.

Flavines were characterized by their solubility in aqueous solutions, insolubility in organic solvents such as petroleum ether, bleaching of the yellow color by the addition of sodium hydrosulfite, reversible on shaking in air, and light absorption in the violet end of the spectrum near 417 mμ.

Carotenoids were extracted, adsorbed on chromatographic columns,

and given chemical and spectroscopic study in accordance with standard methods described elsewhere. (Fox and Pantin, 1941; Scheer, 1940; Fox and Scheer, 1941.)

Results

A preliminary survey, summarized in Table I, shows the distribution of certain of the pigments among various tissues of the two species, and reveals a few arresting facts. In *Paroctopus* we found no sexual dif-

Table I

Distribution of Pigments in the Tissues of *Paroctopus bimaculatus* and *Loligo opalescens*

Species	Tissue	Weight, grams	Carotenoids	Flavines	Melanins
Paroctopus bimaculatus; two specimens, male (742 gm.) and female (778 gm.).	Salivary glands	♂ 3.8	−	−	?
		♀ 3.9	−	−	?
	Crop, stomach and intestine	♂ 4.2	traces	−	−
		♀ 6.4	traces	−	−
	Gills	♂ 6.4	traces	−	−
		♀ 9.1	traces	−	−
	Liver	♂ 13.8	+++	−	−
		♀ 20.1	+++	−	−
	Ink	1 to 2	+	−	+++
	Eyes	♂ 2.1	traces	−	+
		♀ 2.7	traces	−	+
	Gonads	♂ 13.2	traces	−	−
		♀ 2.3	−	−	−
	Pericardial glands	♂ 2.5	−	+	−
		♀ 2.4	−	+	−
	Kidneys*	♂ 1.3	−	···	−
		♀ 1.2	−	··	−
	Heart	♂ 1.3	−	−	−
		♀ 1.3	−	−	−
	Muscle and skin†	♂640	−	?	skin + muscle −
		♀709	−		
	Eggs‡	33.8	traces‡	?	−
Loligo opalescens 7 ♂, 5 ♀	Gills		−	−	−
	Digestive tract		−	−	−
	Ink and sac	2.8 (from 12 animals)	trace?	−	+++
	Eyes	16.5 (from 12 animals)	(+)	?	+
	Accessory nidimental gland	0.9 (from 12 animals)	traces	−	−
	Rest of body (including gonads)		traces?	·····	traces

* The organs, purple in color, yielded quantities of a water-soluble, blood-red pigment discussed in text.

† Acetone extracts of muscle + skin gradually turned a pink-orange color, as did the ground tissue tself. This was not due to either carotenoids or flavines. The pigment was unchanged by acids or salts, but turned yellow in base, and its acetone solution gradually bleached in air.

‡ Laid by another female. Traces of yellow pigment soluble in petroleum ether or $CHCl_3$, gave pink to mauve color in $CHCl_3$ with added $SbCl_3$.

ferences in pigmentation. Here, as in *Loligo,* neither the mature testis nor the ovary or deposited eggs showed more than possible traces of carotenoids. The same was true in gills, digestive tract (minus liver) and eyes, while in the other tissues these pigments were absent. Carotenoids were yielded in considerable quantities by the relatively large liver, however, and were present in appreciable amounts in the ink stored in its sac, attached to the liver but not in direct communication with it. Flavines

were extracted only from the yellowish tripartite pericardial glands (of excretory function), while melanins characterized the ink, iris and skin.

The kidneys of *Paroctopus* were deep purple-red in color and when triturated yielded a blood-red water-soluble pigment. This pigment is insoluble in acetone or alcohol, being reversibly precipitated from water by these solvents. It contains iron and possesses reducing properties. Study of this pigment is being continued, and a more complete account of it will be published later.

Loligo was found to exhibit but little pigment of any kind in most of its tissues. Neither of the mature sexes yielded any pigments in the gonads. Flavines were not detected with certainty, and carotenoids were present in only very slight quantities in the eyes, while the other tissues yielded none or only suspected traces. While the body integument contained very little melanin this pigment was present in both iris and ink in considerable quantities.

Carotenoids in the Liver and in the Ink of the Octopus

The carotenoids were classified according to (1) their behavior in the partition test, both before and after hydrolytic treatment with alcoholic potassum hydroxide: carotenes and xanthophyll esters remain in the upper, or epiphasic layer of petroleum ether, while free xanthophylls migrate to the lower or hypophasic layer of 90 per cent methanol; following hydrolysis, carotenes remain unchanged, xanthophyll esters from the epiphase are split to give free xanthophylls which behave accordingly, while free carotenoid acids are now removed as potassium salts; (2) carotenes were separable one from the other by adsorption on Tswett chromatographic columns of $Ca(OH)_2$, while xanthophylls were resolved into individual pigments on similar columns of $CaCO_3$; (3) finally, absorption spectra of the separate pigments were determined with a Hartridge Reversion Spectroscope or with a Bausch and Lomb Spectrophotometer. Carbon disulfide was employed as solvent in all of the carotenoid observations.

Liver

Carotenes, represented by from two to five colored zones on the chromatogram of the hydrolyzed epiphase, usually showed a fairly prominent red or orange zone near the top, and a yellow zone nearer the bottom of the column. One of the more prominent upper zones gave absorption maxima agreeing closely with those of β-carotene, i.e., 519.5 mμ and 484.4 mμ (Hartridge) and 510 to 512.5 mμ and 482.5 to 485 mμ (B. and

L. spectrophotometer). Another prominent carotene-like pigment appeared above the β-carotene zone, with absorption maxima at from 505 to 507.5 mμ and 480 mμ. At times the shorter wave-length maximum had higher values of 484 to 486 mμ. We did not identify this pigment with any known carotene.

Xanthophylls, present both free and as esters, were represented by considerable quantities of lutein (505 to 508 μ and 474.5 to 479 mμ by Hartridge; 505 to 510 mμ and 477.5 mμ by spectrophotometer), and by lesser amounts of other pigments of the taraxanthin or eloxanthin range of spectra (500 to 505 mμ and 469 to 473 mμ); occasionally a third type was encountered, below the other two on the chromatogram, with absorption maxima not unlike those of antheraxanthin or petaloxanthin, i.e., 512 mμ and 481 mμ (cf. Strain, 1938).

Acidic carotenoids, appearing only after hydrolytic treatment, occurred in both the epiphase and hypophase.[3] They always presented a single, broad and asymmetrical maximum, difficult to determine with precision in the Hartridge instrument, but giving thereby values from 501 to 507 mμ, and, with the spectrophotometer, a broad hump between the values of 500 to 507 mμ, with a slight maximum at about 503 mμ.

Ink

Carotenes were absent.

Xanthophylls, both free and esterified, were found in smaller quantities than in liver tissue. Hydrolytic treatment did not permit subsequent recovery of sufficient quantities for critical spectroscopic study. Inspection of the fresh ink extract, however, revealed, in one instance, absorption maxima similar to those of lutein (510 mμ and 475 mμ). The epiphasic pigments of the ink carotenoids dissolved in carbon disulphide gave an orange solution of esters with maxima at 502.6 mμ and 472 mμ (cf. eloxanthin), while the hypophasic pigments in carbon disulfide failed to yield sharp maxima. Hydrolysis of the epiphase yielded free xanthophylls accompanied by the familiar carotenoid acid. The hypophase yielded, on hydrolytic treatment, an acidic carotenoid, sometimes but not always accompanied by a non-acidic xanthophyll.

In Table II are presented data concerning the relative mass of liver tissue and the range of concentrations of carotenoids encountered in liver and ink. Since the preponderant carotenoid of both liver and ink exhibited, in chemical, adsorptive, and spectroscopic behavior, properties agreeing closely with those of lutein, the concentrations of mixed carotenoids were measured, for purposes of comparison, in "lutein equivalents" with a Bausch and Lomb visual spectrophotometer.

[3] Always hypophasic after hydrolysis of the extract.

The combined weight of liver and ink is seen to vary between 1.86 per cent and 3.29 per cent of the total body weight, with a mean value of about 2.7 per cent. We were rarely able to recover more than one or two grams of the ink, which represented from 5 to 11 per cent of the combined weight of liver, ink-sac and ink.

The concentration of carotenoids (in lutein equivalents) in liver tissues ranged between 1.2 and 8.3 mg. per 100 gm. with a mean value of about 3.5, in freshly excised tissue from normal animals, i.e., excluding the relatively low value of 0.8 given by the autolyzed liver of animal No. 7. The ink of freshly caught specimens yielded appreciable amounts of

TABLE II

Concentrations of Carotenoids in Liver and Ink

Animal (sex)	Weight, grams	Liver + ink weight, grams	Liver and ink % of total body weight	Total carotenoids (as lutein) mg./100 g.
1 ♂	742	13.8	1.86	2.4
2 ♀	778	20.1	2.58	2.1
3 ♂	716	20.6 (liver)	3.19	1.2
		2.3 (ink)		0.70
4 ♂	721	18.9 (liver)	2.77	3.9
		1.1 (ink)		0.55
5 ♂ (starved)	820	25.4 (liver)	3.29	.....[1]
		1.6 (ink)		0.0
6 ♂ (immature)	493	12.7	2.57	8.3[2]
7 ♂	623	15.1	2.42	0.82[3]

[1] Very little of the pigment in this specimen was carotenoid, having evidently been altered by starvation.

[2] Quantity of carotenoids unchanged by incubating toluene-preserved macerated liver at its natural pH (5.42) for 44 hours at 37° to 40° C.

[3] Liver + ink autolyzed by placing in closed flask in presence of a few drops of $CHCl_3$ for 16 days at room temperature.

carotenoids showing values of from 0.55 to 0.7 mg. per 100 gm. (mean value from two determinations 0.62 mg. per 100 gram) while the ink of one specimen (No. 5) which had been starved for two or three weeks was found to be completely lacking in carotenoids.

DISCUSSION

Regarding the chief role of the liver-pancreas in cephalopods, Jordan (1929) states that it possesses only a secretory function, elaborating digestive enzymes which are passed into the gut during feeding.

In its lack of absorptive function, and its secretion of extracellular enzymes, the liver of cephalopods differs markedly from that of lamellibranch mollusks (see Yonge, 1931). The California mussel, a typical

phytoplankton feeder, passes great amounts of minute plant plastids into its digestive diverticulum, or "liver," whence certain cells engulf and absorb food, passing the undigested residues back into the digestive tract (Fox et al., 1936; Scheer, 1941, and unpublished observations on voiding of feces during early starvation). It would appear obvious that the liver of the mussel receives its rich supply of carotenoid pigments in a direct way from the plant material taken in. The liver of the octopus, on the other hand, probably receives its supply of carotenoids through the blood stream.

Very little seems to be known of the physiology of ink secretion in cephalopods, or of the biochemistry of the ink itself, beyond its rich melanin content. Its carotenoid content provokes much thought. We are aware of the loss of carotenoids from ectoderm in various ways, such as the sloughing of skin, the growth of feathers in certain birds, the discharge of ear-wax in cattle, and the presence of such pigments in the cores of solid materials secreted from the femoral pits of iguanas (unpublished data). Save for the storage of carotenoids in egg-yolk of many oviparous vertebrates and invertebrates, and the secretion of such pigments in the milk fat from mammary glands, the discharge of carotenoids from an internal structure to the outside would appear to be rather unique.

Carotenoids of *Paroctopus* disappeared from both liver and ink in a starved specimen, yet were reduced in amount only very slowly on autolysis of the liver at room temperature, or by incubation of the ground tissue at 37° C.

Clearly, a thorough investigation of the physiological role of the liver is called for, likewise a biochemical study of the ink and its relationship to the metabolism of the animal as a whole, with particular reference to the liver.

Summary

The kinds and tissue-distribution of various pigments in the cephalopods *Paroctopus bimaculatus* and *Loligo opalescens* are described and discussed. Melanins, stored in quantity in eyes and ink of both species, are far more plentiful in the integument of the octopus than in that of the squid.

Flavines were detected with certainty only in the pericardial (excretory) glands of the octopus. This species also yielded, from its kidneys, a water-soluble, iron-containing red pigment with reducing properties.

Carotenoids were present only in traces in any of the organs of the squid. This was likewise true of the octopus, save in the liver-pancreas of this species, which contained a variety of carotenoids in amounts of

3.5 mg. (lutein equivalents) per 100 grams of moist tissue (average value). Ink from the sac adjacent to the liver contained 0.55 to 0.70 mg. lutein equivalents per 100 grams.

In the liver of *Paroctopus,* free and esterified xanthophylls, predominantly of the lutein class, were accompanied by smaller amounts of other xanthophylls, β-carotene and an additional unfamiliar carotene, and a unique carotenoid acid, appearing on hydrolysis, not identical spectroscopically with astacene or with the metridene of Fox and Pantin.

The ink of this species yielded no carotenes, but free and esterified xanthophylls and (on hydrolysis) a carotenoid acid similar to respective compounds recovered from the liver. Whilst starvation brought about the gradual disappearance of carotenoids from liver and ink, autolysis of the liver or incubation of its ground tissues resulted in only slow loss of the pigments.

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